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1. **Introduction**

Nasopharyngeal carcinoma (NPC) is consistently associated with the Epstein-Barr virus (EBV), and is characterized by marked geographic and population differences in incidence[1,2]. As EBV infection is almost ubiquitous in the world, the development of cancer in a subset of infected population suggests that there are certain contributing factors in those who develop NPC. Indeed, studies of EBV and its associated tumors suggested specific interactions between environmental, genetic, and viral factors.

2. Epidemiology

NPC develops extremely high incidence in southern China and Southeast Asia. The recent reported incidence of NPC among men and women in Hong Kong (geographically adjacent to Guangdong province) was 20 to 30 per 100,000 and 15 to 20 per 100,000 respectively[1]. There is also increased incidence in northern Africa and the Inuits of Alaska[3], but it is an uncommon disease in most countries, and its age-adjusted incidence for both sexes is less than 1 per 100,000[2]. The incidence of NPC remains high among Chinese who have immigrated to other parts of Asia or North America, but is lower among Chinese born in North America than in those born in southern China[4,5]. Thus, it is suggested that genetic, ethnic and environmental factors could have some roles in the development of NPC.

3. Pathology

Regardless of geographic distribution or racial background, EBV is consistently detected in NPC[6]. Again, the consistent association with EBV and the remarkable patterns of incidence suggests the contribution of other co-factors to the development of this type of NPC.

The tumor presents with varying degrees of differentiation and has been classified by the World Health Organization into three categories[7]. Squamous cell carcinoma, WHO I tumors, are highly differentiated with characteristic epithelial growth patterns and keratin filaments. Non-keratinnizing, WHO II carcinomas, retain epithelial cell shape and growth patterns. Undifferentiated carcinomas, WHO III, do not produce keratin and lack a distributive growth pattern. Recently, based on the etiological view point, an alternative, simpler classification was proposed and this divided NPC into two histological types, namely squamous cell carcinomas (SCCs) and undifferentiated carcinomas of the nasopharyngeal type (UCNTs)[8]. This classification correlates with Epstein Barr virus serology tests. Those patients with SCCs have a lower EBV titer, while those with UCNTs have elevated titers. This classification is more applicable for epidemiological research and has also been shown to have a prognostic value. The UCNTs have a higher local tumor control rate with therapy, and a higher incidence of distant metastasis[9,10]. In North America, tumor histology in 25% of patients is

Type I, 12% Type II, and 63% Type III while the corresponding histological distribution in southern Chinese patients is 2%, 3%, and 95% respectively[11,12].

Premalignant lesions

In contrast to many cancers including head and neck cancers, premalignant lesions such as dysplasia or carcinoma in situ (CIS) are not usually detected in the development of NPC. The very low incidence of coexistent nasopharyngeal intraepithelial neoplasia with invasive cancer, approximately 3%, and the follow-up data all indicated a rapid progression of the initiated cell through the sequence of the dysplasia, CIS, and invasive cancer[13]. Thus, the biologic behavior of NPC is quite distinct from the malignant transformation and progression of head and neck carcinoma other than NPC, or cancer of the uterine cervix where premalignant dysplasia and CIS of the head and neck cancer and cervix cancer may persist for many years. EBV has been detected in all of the samples of preinvasive carcinoma (CIS) with EBERs expressions in the majority of cells. Moreover, expression of EBNA1, LMP1, LMP2A, and transcripts from BamHI A was detected in all of the premalignant lesions. A single restriction enzyme fragment study showed that preinvasive lesions contain clonal EBV and represent a focal cellular growth that developed from an EBV infected cell. The rarity of preinvasive lesions and the more frequent detection of CIS concomitant with invasive cancer suggest that the clonal infection by EBV is an early event in the development of NPC.

In other similar studies, example of milder dysplasia were identified that contained EBV in only a portion of cells[14]. This finding suggested that EBV infection occurred after some stimulus affected cell growth. Usually, EBV infection in epithelial cells is thought to result in viral replication. The fate of cells in which herpesvirus replicate is not malignant transformation but cell lysis. Thus, it is also possible that some preceding genetic change affects viral infection such that a latent EBV infection is established with expression of potent genes such as LMP1 and LMP2 that significantly affect cell growth. A loss of the p16 tumor suppressor gene was identified in early, EBV-negative lesions[15]. The expression of EBV transforming proteins, in combination with p16 loss, could result in rapid progression to invasive malignancy.

4. Gene expression

i) EBV gene expression and clinical feature in NPC

Three EBV-encoded genes, the EBV-encoded small nuclear RNAs (EBERs), latent membrane protein (LMP)1 and 2A are detected in NPC samples. Many studies have identified abundant transcription of in EBV genome positive NPC cells[16,17]. LMP1 upregulates all steps of metastasis, such as down regulation of cell-cell adhesion, degradation of basement

membrane and interstitial stroma, upregulation of tumor cell motility, and angiogenesis. Upregulation of metastasis related steps in the early stage reflect highly metastatic feature of NPC[18-24]. In fact, most common clinical symptom is neck mass[25]. The role on LMP2A in NPC is still obscure.

ii) Tumor suppressor genes

As with all cancers, the development of NPC involves the loss of tumor suppressors. However, the mechanisms of losing tumor suppressors in NPC seem somewhat unique.

a) High Levels of p53 Are Found in NPC

p53 is a hallmark tumor suppressor that induces cell cycle arrest in response to DNA damage. In most head and neck cancers, low levels of p53 are due to mutations[26]. However, p53 in NPC does not follow this classic pattern. NPC cells have increased levels of p53[27]. with high LMP1 levels correlating with higher p53 expression[28,29]. p53 mutations are relatively rare in NPC, so the vast majority of expressed p53 is wild type[30]. The wild-type p53 fails to induce apoptosis in NPC because it is inactivated through 2 mechanisms—loss of p14 and excess DN-p63. p14 maintains p53 stability by inhibiting its proteolysis[31].; in NPC, p14 levels are low via promoter hypermethylation,99 thus allowing for more efficient p53 degradation. p63 is actually a homologue of p53 with similar DNA binding sequences to p53[32]. There is a mutated version of p63 in NPC, called DN-p63, which lacks the N-terminal transactivation domain needed to activate apoptosis[33]. The DN-p63 isoform binds the normal p63 (and p53) DNA sequences, thus preventing normal p63 or p53 from binding, but fails to induce apoptosis because of loss of the N-terminal sequence. The reason for high p53 levels in NPC is unclear. High levels of normal p53 may be advantageous to NPC development because tumor cells with normal p53 are immune to JNK-induced apoptosis[34]. Alternatively the increased wildtype p53 may simply be the natural response to EBV infection[35]. In light of this, it appears that the loss of p53 is not critical for NPC development, suggesting that inactivation of other tumor suppressors is likely required for NPC tumorigenesis.

b) p16 Activity Is Decreased

p16 is a cyclin-dependent kinase inhibitory protein (CKI) that suppresses activity and is frequently inactivated in cancer[36]. The normal function of p16 is to suppress cdk4, an enzyme that controls the G1/S checkpoint by negatively regulating cyclin D1 activity. Therefore, loss of p16 results in cyclin D1 overactivation and subsequent increases in G1/S phase transitions[37]. In head and neck cancers, including NPC, the majority of head and neck cancers exhibit low p16 levels with high pRb levels[38,39]. In HNSCC, the most common mechanism

of p16 inactivation is homozygous deletion of the gene followed by hypermethylation of the gene[40]. Similarly, NPC cell lines have low levels of p16 secondary to hypermethylation of the p16, but this epigenetic alteration may be mediated by LMP1-induced c-Jun/JunB heterodimer formation, which activate DNA methyltransferase[41]. Additionally, LMP1 inactivates p16 by inducing cytoplasmic accumulation of E2F4/5 and Ets2, which are nuclear proteins required for normal p16 activity. Ets2 is a key transcription factor for p16 expression and LMP1 directly causes its translocation from the nucleus to the cytoplasm[42]. E2F4/5 are proteins required for the activation of p16-induced cell cycle arrest in G1, and their nuclear localization is dependent on binding to nuclear pRb[43]. LMP1 induces the translocation of E2F4/5 from the nucleus into the cytoplasm by causing E2F4/5 to dissociate from pRb proteins. LMP1 further promotes cytoplasmic accumulation of Ets2 and E2F4/5 by mediating their binding to a nuclear export protein called chromosome maintenance region 1 (CRM-1). About two thirds of NPCs exhibit low p16 levels, indicating that p16 downregulation is not critical to NPC development. However, the absence of p16 is still important to NPC. Patients with NPC tumors with low p16 levels have a worse prognosis because this is associated with decreased radiosensitivity and higher rates of tumor recurrence[44,45]. The reason for this may be that the greatest radiosensitivity in cells is just prior to DNA synthesis and loss of p16 increases the number of cells in S phase.146 There is some data that pretreatment of NPC patients with p16 gene therapy before radiation can improve outcomes; the presumed mechanism behind this is that by normalizing p16 levels, the cell cycle is slowed at the G1/S checkpoint and the number of cells in G1 increased[46]. However, further clinical trials are needed to evaluate the effectiveness of this treatment.

5. STAGING AND TREATMENT

i) Prognostic factors

The tumor, nodes, and metastases (TNM) staging for NPCs is the most important prognostic factor[47]. Indeed, most other known prognostic factors are directly or indirectly related to the extent or bulk of the tumor.

A study reported in 1992 showed that the tumor's histological type and the radiotherapy dosage and coverage were also significant independent prognostic factors[48]. The histological type, WHO Type I patients frequently seen among the Caucasian population were found to be associated with adverse prognosis[49].

A large variation of tumor volume is present in T stages and primary tumor volume represents an independent prognostic factor of local control. Its validity has been confirmed in patients with T3 and T4 tumors, and there is an estimated 1% increase in risk of local failure for every 1 cm^3 increase in primary tumor volume[50].

Amount of circulating EBV DNA in NPC, estimated to reflect the tumor load, has a positive correlation with disease stage. It has been shown to have prognostic importance[51].

ii) Radiotherapy

NPC, most of which has wild-type p53, is highly radiosensitive. Thus, radiotherapy (RT) plays a central role in the treatment of all stages of NPC without distant metastases. The patients with distant metastasis may be treated with RT in case of controlling local disease has benefit for improve the patients' QOL.

Conventional 2dimensional (2D) RT successfully controlled T1 and T2 tumors in between 75% to 90% of cases, and T3 and T4 tumors in 50% to 75% of cases[52,53]. Nodal control is achieved in 90% for N0 and N1 cases, but the control rate drops to 70% for N2 and N3 cases[52]. As interrupted or prolonged treatment reduces the benefits of RT, every effort should be made to maintain the treatment schedule[54]. Because of the high incidence of occult neck node involvement, prophylactic neck radiation is usually recommended[55]. Good loco-regional control should be the prime objective of treatment, as loco-regional relapses represent a significant risk factor for the development of distant metastases[56].

For T1 and T2 tumors, a booster dose using intracavitary brachytherapy improved tumor control by 16%[53]. However, it is probably better to reserve stereotactic radiosurgery for the treatment of persistent and recurrent NPCs, because of the undesirable side effects associated with hypofractionated treatment[57-59]. RT for NPC is challenging because the nasopharynx is anatomically surrounded by an array of radiosensitive structures such as the brain stem, spinal cord, pituitary-hypothalamic axis, temporal lobes, eyes, middle and inner ears, and parotid glands. As NPCs tend to infiltrate and spread towards these normal organs, the irradiation target volumes in NPC are very irregular. For patients with early disease, since they have a good chance of survival, radiation toxicities in these even non-critical structures would affect the quality of life of survivors. However, for patients with locally advanced disease like those with skull base or intracranial extension, the challenge lies in achieving adequate tumor controlling dose to the primary tumor with sparing the critical organs.

The major limitations of conventional 2D radiotherapy for NPC can now be overcome with 3 dimensional (3D) conformal RT and intension modulated radiotherapy (IMRT)[60,61]. IMRT is an advanced form of 3D conformal radiotherapy, conforming high dose to tumor while conforming low dose to normal tissues. The ability of IMRT to deliver a more conformal radiation dose to the target area and spare surrounding structures seems to decrease the toxicity of chemoradiotherapy[62]. A good therapeutic result can be achieved by distributing a high dose to the tumor while keeping down normal tissue complications by reducing radiation dose to normal tissues. Some researchers reported excellent local control of more than 90% in NPC

achieved with IMRT[63], even among patients with advanced T3-4 diseases[64]. Reports also shown preservation of salivary function and improve quality of life of survivors after IMRT[65,66]. A recent multicentre study also showed that the excellent 90% local control rate with IMRT as reported from single institutions are reproducible in multi-institutional setting[67]. Thus, IMRT has gradually been considered as the new standard RT for NPC.

iii) Chemotherapy

As the development of RT achieves good local control, distant metastatic failure become the pattern of recurrence, especially among those with loco-regionally advanced disease, which could hardly be controlled with conventional 2D RT. As radiosensitivity well correlates with chemosensitivity, NPC is also chemosensitive. Many clinical studies investigated advantage of chemotherapy for NPC. However, most of the early studies were non-randomized and the results are not conclusive[68-70]. So far, there were only 5 reported randomized trials on combined chemotherapy and RT for NPC[69,71-74]. In the early randomized trials, induction chemotherapy is the most often studied combination[69,72,74]. The rationale for induction chemotherapy is to reduce tumor load of loco-regional disease before start of RT and also early use of systemic treatment for eradication of micro-metastases. The International Nasopharyngeal Carcinoma Study Group[69], using combination of bleomycin, epirubicin and cisplatin, showed a significant improvement of disease-free survival, but, did not show improvement of overall survival. This discrepancy may be due to the increased treatment-related death among patients on induction chemotherapy compared to RT alone (8% vs 1%).

A pivotal study was reported by the Head and Neck Intergroup in 1998, using concurrent RT with cisplatin (100 mg/ m^2 day1, 22, 43) followed by adjuvant cisplatin and 5-fluouracil (5-FU) (cisplatin 80 mg/m^2 day1 and 5-FU 1,000 mg/ m^2 /day, day1-4, 4 weeks cycles for 3 cycles)[73]. Compared with RT alone, chemoradiation (CRT) significantly improved progression free survival and overall survival. The pattern of disease failure showed reduction of both loco-regional and distant failure with CRT.

After report of the Intergroup 0099 Study, randomized trials using similar design were performed in endemic regions in Asia, to validate the Intergroup results. Three randomized trials were subsequently reported from Hong Kong[75], Singapore[76], and China [77] respectively. The HK study, using the same chemotherapy regime as the Intergroup study 0099, showed improved local control with CRT. However, There was no significant difference between distant control and overall survival[75]. On the other hand, the Singapore and China studies both used a variation of dosing of cisplatin from the 0099 regimes, using lower dose of

cisplatin more frequently, showed significant reduction of distant metastases and improvement of both disease-free and overall survival with CRT[76,77].

Although some reports resulted in unfavorable feasibility, it is now generally accepted that Intergroup 0099 is feasible to the NPC in Asian, including endemic and non-endemic areas. The 0099 study showed that concurrent CRT was the most efficacious. There were 3 randomized trials that evaluated adjuvant chemotherapy all of which showed negative results[71,78,79]. Thus, there is still debate on the efficacy of the adjuvant chemotherapy in the Intergroup 0099. Compared to the Intergroup 0099 Trial, alternating CRT in Aichi Cancer Institute was characterized by a decreased total dose of CDDP (540 mg/m2 in the Intergroup 0099 vs. 300 mg/m2 in Aichi), a shorter treatment period (130 days in the Intergroup 0099 vs. 83 days in Aichi), a higher treatment completion rate (55%: 43 of 78 cases in the Intergroup 0099 vs. 80%: 70 of 87 cases in Aichi), and a higher 3-year overall survival rate (78% in the Intergroup 0099 vs. 92% in Aichi)[80]. Although the final therapeutic value of this alternating CRT cannot be currently evaluated, this method can be used in a controlled clinical trial in the future to compare therapeutic results with those of the concurrent CRT.

Impairment of quality of life, mainly attributable to radiation is a serious problem for survivors of NPC treated with radiotherapy or CRT[81,82]. The use of chemotherapy in more advanced cases adds to the side effects, which include ototoxicity associated with cisplatin.

iv) Managenent of residual or recurrent disease

Despite concomitant CRT generated remarkable improvement in the management of nasopharyngeal carcinoma, some patients still developed loco- regional recurrence presenting as persistent or recurrent tumor. Early detection is essential for any form of salvage therapy to be successful. Generally, planned neck dissection is not recommended. If a complete remission has been obtained in the neck, no salvage surgery is considered necessary and if a metastatic mass in the neck does not disappear completely, a neck dissection is performed.

FDG-PET, definitely powerful for detection of primary nasopharyngeal carcinoma and in particular, has a high-negative-predictive value and is considered reliable to evaluate the response to treatment, is not always superior to other imaging studies such as computed tomography or MRI in detecting residual or recurrent disease. Residual or recurrent tumor in the cervical lymph nodes after radiotherapy is notoriously difficult to confirm, as in some lymph nodes only clusters of tumor cells are present [83]. Thus, sometimes the diagnosis was only confirmed after salvage surgery. The other argument is the type of dissection. Wei et al analyzed serial sections in a series of whole-neck dissection specimens and found that all levels of the neck compartment had the potential of being involved with level II being the most common (53%). They also found the incidence of extranodal extension to be 84% in the patients

with extensive recurrent or persistent disease in the neck, and concluded that RND with additional brachytherapy is a treatment of choice for such selected patients [83]. On the contrary, Khafif advocated that the neck dissection should be tailored to the patient and although the procedure of choice is currently an RND due to lack of data, one may consider less aggressive procedures in selected patients with limited persistent disease [84]. Therefore, as is the case with the management of recurrent and residual neck disease in the other head and neck cancers, trends in the salvage neck dissection for residual neck mass has still been controversial.

Aggressive salvage treatment for locally recurrent NPC is warranted, especially when the disease is confined to the nasopharynx [85]. Even for patients with synchronous loco-regional failures, aggressive treatment should be considered for selected patients. There are three popular approaches for nasopharynx, transoral (transpalatal), transmaxillar (maxillar swing), and endoscopic approaches. Close evaluation for the indication of surgical treatment and approach to the nasopharynx. Patients whose local disease was treated by surgical resection had a 3-year local control rate of 71% compared with 38% by reirradiation using brachytherapy or external radiotherapy. For regional disease, the 3-year nodal control rate after radical neck dissection was 65% compared with 24% by reirradiation[86].

Immunotherapy and anti-viral drug, targeting EBV gene products, are not applicable for all recurrent cases, either. It is desired to establish the way to identify the patients who are expected to have benefits from these treatments [87-89].

6. Conclusion

There have been remarkable progress in the understanding of molecular mechanism of EBV-mediated carcinogenesis and treatment modality in nasopharyngeal carcinoma. However, many issues such as “ How EBV infect to nasopharyngeal mucosa?” “Why racial/ethnic predisposition exist?” How EBV-mediated carcinogenesis triggered in healthy carriers?”, have been unsolved. The cue to answer these subjects would be found by physician scientists specialized in Otolaryngology-Head and neck surgery through clinical observation.

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